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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1656	

DATE MAILED: 01/04/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/815,533	ARINI ET AL.	
	Examiner	Art Unit	
	David J. Steadman	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *31 May 2005 and 12 September 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *93-111* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *93-111* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

[1] The Art Unit location of your application in the USPTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Art Unit 1656.

[2] Claims 93-111 are pending in the application.

[3] Applicant's amendment to the claims, filed on 9/12/2005, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

[4] Applicant's amendment to the specification, filed on 9/12/2005, is acknowledged.

[5] Applicant's arguments filed on 9/12/2005 have been fully considered and are deemed to be persuasive to overcome some of the rejections and/or objections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

[6] The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Claim Objections

[7] Claim 101 is objected to in the recitation of "pH form 5.0 to 5.8" in line 3. "Form" should be replaced with "from."

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

[8] Claims 93-111 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 93 (claim(s) 94-101 dependent therefrom), 102 (claim(s) 103-110 dependent therefrom), and 111 are indefinite in the recitation of "derivatives" because neither the specification nor the claims defines the term and it is unclear as to the scope of compounds that is encompassed by the term. It is suggested that applicant clarify the meaning of the term.

Claim Rejections - 35 USC § 102

[9] The rejection of claim(s) 93-100 and 102-109 under 35 U.S.C. 102(b) is withdrawn in view of applicant's argument, pointing out that, during the "main incubation," "[t]he incubation liquids are exchanged every 2 ~ 3 days or so" (p. 8, bottom). According to the Rule 132 Declaration filed on 5/15/2003 (p. 2, Figure 2), a culture time greater than 3 days is required to achieve the recited level of two-chain urokinase. As such, the reference of Okabayashi et al. does not anticipate the claims because Okabayashi et al. does not *specifically* teach a culture time of greater than 3 days.

Claim Rejections - 35 USC § 103

[10] Claim(s) 93-100, 102-109, and 111 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okabayashi (Japanese Patent No. H01-25749, cited in the 1/27/2005 Office action) in view of Okabayashi et al. (*Cell Struc Func* 14:579-586, cited in the 12/8/2003 IDS), Palermo et al. (*J Biotechnol* 19:35-48), Chang et al. (*Free Radic Res* 30:85-91), Li et al. (*Chin J Biotechnol* 7 :113-120), Cheng et al. (*Chin J Biotechnol* 9 :151-159, cited in the 4/26/2001 IDS), and Zang et al. (*Biotechnol* 13:389-392, cited in the 10/6/2003 Office action). The claims are drawn to processes for recombinant production of tc-uPA by culturing a mammalian cell line transfected with a urokinase cDNA in the presence of butyrate and culturing until 95% of the total urokinase is tc-uPA.

At the time of the invention, the use of sodium butyrate to induce recombinant gene expression was well-known in the art. For example, Palermo et al. teaches a detailed analysis of the production of tissue-type plasminogen activator (different from uPA) using a Chinese hamster ovary (CHO) cell line as an expression host in a culture medium comprising 5 mM sodium butyrate (p. 35, abstract and p. 40, bottom). Palermo et al. teaches the presence of butyrate in the culture medium over a 72-hour time period enhanced the induction of tPA by 2 to 9-fold (p. 35, abstract and p. 41, Table 1) and additionally had the effect of reducing the growth rate of the cells, thus minimizing the need to split cells during production and increasing the productive life of the cultures and reducing the quantity of cells needed to generate the required amount of protein (p. 35, abstract and p. 43, middle). Palermo et al. teaches that “butyrate-mediated induction

of gene expression is likely to be a general phenomenon, showing little preference for promoter or gene" (p. 45, middle). Also, the references of Okabayashi and Okabayashi et al. teach a method for the production of recombinant urokinase by culturing a CHO cell line transfected with an expression vector encoding pro-urokinase in a serum-free medium comprising butyrate at a concentration of 0.1 to 10 mM at a temperature of 20 to 37 degrees Celsius for a period "2 to 3 days or so," and recovering the urokinase from the medium (see particularly pp. 8 and 17-25 of Okabayashi). Okabayashi teaches that the presence of butyrate in the culture medium increased the level of expressed urokinase by 2-3 fold.

Neither Palermo et al. nor Okabayashi et al. *specifically* teaches culturing cells in the presence of butyrate for greater than 3 days. However, culturing CHO cells in the presence of butyrate for greater than 3 days for recombinant protein production was well known in the art at the time of the invention. For example, the reference of Chang et al. teaches increased biosynthesis of recombinant EPO in a CHO cell line cultured in the presence of sodium butyrate over a 4 day period (p. 85, abstract). While Chang et al. teaches treatment of cells undergoing rapid cell division with sodium butyrate induces apoptosis, leading to decreased cell viability, the reference teaches the decrease in cell viability can be overcome by simply adding N-acetylcysteine (NAC) to the culture medium (p. 88, Figure 3), which has the added effect of significantly enhancing the production of recombinant protein over the 4 day period (p. 89, Figure 4).

Also, at the time of the invention, methods for recombinantly producing urokinase using a Chinese Hamster Ovary (CHO) host cell and a culture time of at least as long as

5 days were well-known in the art. For example, Li et al. teaches the recombinant production of urokinase using a CHO host cell transfected with pro-urokinase cDNA (p. 115), cultured for up to 5 days (p. 117, middle). Also, Cheng et al. teaches a method similar to that of Li et al. with stable secretion of 180-200 IU/mL of urokinase during a 6-day cultivation (p. 157, middle and p. 158, Figure 5). Further, Zang et al. teaches a method for the production of recombinant urokinase in CHO cells using a serum-free cell culture medium and a culturing time of up to five days is sufficient for a near-maximum concentration of urokinase (see page 390, Figure 3A). Zang et al. teaches at least one advantage of using serum-free medium in the recombinant production of urokinase is a reduction in the likelihood of introducing transmissible contaminants (page 389, left column).

Therefore, at the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Okabayashi, Palermo et al., Chang et al., Li et al., Cheng et al., and Zang et al. to practice the method for urokinase production as taught by Okabayashi for a time greater than 3 days, including 120 hours. One would have been motivated to practice the method of Okabayashi for a time greater than 3 days, including 120 hours in order to obtain an increased yield of urokinase using the existing cell culture. One would have a reasonable expectation of success for practicing the method of Okabayashi for a time greater than 3 days, including 120 days because of the results of Okabayashi, Palermo et al., Chang et al., Li et al., Cheng et al., and Zang et al. Therefore, claims 93-100, 102-109, and 111,

drawn to processes for the recombinant production of tc-uPA as described above would have been obvious to one of ordinary skill in the art.

[11] RESPONSE TO ARGUMENT: Addressing the reference of Okabayashi, applicant argues: 1) the incubation time in the presence of butyrate ranges from 6-24 hours; 2) the fibrin plate method for urokinase detection does not discriminate between sc-uPA and tc-uPA and identifies the total amount of urokinase; 3) the expected product of Okabayashi would be pro-UK or a mixture of active and inactive UK; 4) the actual cultivation of cells in the presence of butyrate was only 3 days; and 5) claim 111 requires recovery of tc-uPA, claim 102 requires a 120 hour culture time, and claim 105 requires a serum free culture process.

Applicant's argument is not found persuasive. While it is acknowledged that Okabayashi does not teach a culture time in the presence of butyrate greater than 3 days (although one would recognize that it is suggested by the disclosure of "2 ~ 3 days or so..." the *combination* of references clearly teaches a culture time greater than 3 days in the presence of butyrate, including 120 hours. Okabayashi also teaches recovery of the produced urokinase and as with Zang et al., teaches a serum free culture process. Applicant argues that one of ordinary skill in the art would expect the method of Okabayashi to produce only pro-UK or a mixture of active/inactive UK. However, an ordinarily skilled artisan at the time of the invention would have recognized that the polypeptide produced by Okabayashi or Okabayashi et al. is urokinase, not pro-urokinase. See also the references of Li et al. and Cheng et al. In fact, Okabayashi teaches that the UK activity of the produced protein was measured, not the pro-UK

activity. The examiner maintains that by practicing the method of Okabayashi for a time greater than 3 days, *e.g.*, 5 days, as suggested by the combination of the references, one of ordinary skill in the art would have produced tc-uPA in the desired amount of 95%. Applicant has yet to present any evidence to the contrary.

[12] Claim(s) 101 and 110 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okabayashi in view of Okabayashi et al., Palermo et al., Chang et al., Li et al., Cheng et al., and Zang et al. as applied to claims 93-100, 102-109, and 111 above and further in view of Paques (US Patent 5,156,967, cited in the 6/3/2004 Office action) and Frommer et al. (*Appl Microbiol Biotechnol* 39:141-147, abstract only). Claims 101 and 110 are drawn to the processes of claims 93 and 102, respectively, wherein the culture medium is acidified with an acid of pH from 5 to 5.8 and optionally a non-ionic detergent is added and the culture medium is filtered.

Okabayashi, Okabayashi et al., Palermo et al., Chang et al., Li et al., Cheng et al., and Zang et al. disclose the teachings as described above. None of the references discloses acidifying the culture with an acid of pH from 5 to 5.8 and optionally a non-ionic detergent is added and the culture medium is filtered.

Paques teaches a method for sterilizing a solution of UK for therapeutic use by adjusting the pH of the solution to 5.5 with an acid, heating the solution, and sterilizing the solution by filtration (column 4, top).

Frommer et al. teaches that cell cultures may be contaminated with viruses.

Therefore, at the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Okabayashi, Okabayashi et al.,

Palermo et al., Chang et al., Li et al., Cheng et al., Zang et al., and Paques to practice the method of Okabayashi using a culture time greater than 3 days, *e.g.*, 120 hours, and sterilize the harvested culture medium by the method of Paques. One would have been motivated to sterilize the culture medium by the method of Paques in order to remove pathogenic viruses that may be present in a culture medium as taught by Frommer et al. One would have a reasonable expectation of success for practicing the method of Okabayashi using a culture time greater than 3 days, *e.g.*, 120 hours and sterilizing the resulting harvested culture medium by the method of Paques because of the results of Okabayashi, Okabayashi et al., Palermo et al., Chang et al., Li et al., Cheng et al., and Zang et al. and Paques. Therefore, claims 101 and 110, drawn to processes for the recombinant production of two-chain UK as described above would have been obvious to one of ordinary skill in the art.

[13] RESPONSE TO ARGUMENT: Applicant argues Pacques refers to pasteurization of urine and would not have applied the method to a recombinant cell culture.

Applicant's argument is not found persuasive. Pacques teaches the method is for sterilization of “a solution of urokinase.” While it is acknowledged that Pacques teaches urine as a representative example of “a solution of urokinase,” there is no teaching in Pacques or any prior art of record that would suggest that the method is limited to sterilization of urine and cannot be equally applied to other solutions of urokinase. As the method is shown to inactivate pathogenic viruses and Frommer et al. teaches that such may be present in a cell culture, one of ordinary skill in the art at the time of the invention would have recognized that the method would apply to cell culture.

Citation of Relevant Art

[14] The art made of record and not relied upon is considered pertinent to applicant's disclosure. Kim et al. (*Biotechnol Prog* 20 :1788-1796) teaches a method for recombinant production of urokinase using a CHO host cell line in the presence of butyrate and culturing the cells for as many as 22 days. Kim et al. included aprotinin in the culture medium, and consequently, single-chain urokinase was produced.

Conclusion

[15] Status of the claims:

- Claims 93-111 are pending.
- Claims 93-111 are rejected.
- No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is 571-272-0942. The examiner can normally be reached on Mon to Thurs and alternate Fri, 7:30 am to 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Kerr can be reached on 571-272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David J. Steadman, Ph.D.
Primary Examiner
Art Unit 1656